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WATER DRIVE STUDIES ON HYPOPHYSECTOMIZED EFTS OF DIEMYCTYLUS VIRIDESCENS. PART I. THE ROLE OF THE LACTOGENIC HORMONE

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It is well known that following metamorphosis the eastern, spotted newt *Diemyctylus viridescens* passes into a terrestrial or red eft stage which lasts from three to four years before the animals migrate to water where they become sexually mature. In certain parts of Long Island and in the Woods Hole area, however, Noble (1926, 1929) found that the eft stage failed to develop. There has been considerable interest in the role played by the endocrine glands in the events associated with the migration of efts from land to water. Adams (1932) was able to induce adult skin texture and pigmentation in efts injected with an anterior lobe preparation (phyone), while Dawson (1936) showed that pituitary preparations administered to efts brought about the maturation of the lateral line system. The studies of Reinke and Chadwick (1939) demonstrated that efts receiving implants of whole adult pituitaries or anterior lobes voluntarily migrated to water from 2 to 6 days following treatment. The test animals acquired a smooth, moist skin and showed a tendency toward the olive pigmentation of the adult. In certain cases keeling of the tail was evident after an extended period. The thyroids showed some stimulation as the result of the implants but the gonads remained unaffected. Molting usually occurred on the second to fourth day following implantation. Gonadectomized efts molted and entered water from 4 to 8 days after implantation of adult pituitaries according to Reinke and Chadwick (1940). Thyroidectomized individuals and animals which had been both thyroidectomized and gonadectomized were forced to water following similar treatment, although in these cases molting was abnormal with pieces of cornified epithelium sloughing off in patches after the animals had assumed the aquatic habitat.

The failure of thyroidectomized efts to undergo a normal molt is understandable in the light of investigation by Adams and her co-workers. Adams *et al.* (1932) and Adams and Grierson (1932) have shown that a pituitary-thyroid relationship is necessary for proper molting. Changes in cutaneous circulation, rather than the stimulation of secretion of the cutaneous glands, may be of major importance to the molting process. According to Chadwick (1948), however, the thyroid exerts a direct effect on molting by the stimulation of the inter-papillary skin glands. An increased incidence in molting, noted by Chadwick and Jackson (1948) following the injection of efts with prolactin, may have been due to the stimulation of cell division in the epidermis.

Chadwick (1940a) induced water drive in efts ranging from 60 to 95 mm. in length with injections of Antuitrin G (Parke Davis). This preparation was less effective than implants of adult newt pituitaries, as it failed to induce water drive in smaller animals or those which had been thyroidectomized. Prolactin was identified with the active water drive principle of the anterior lobe by Chadwick (1940b). Injections of 14 to 20 mg. of prolactin (Eli Lilly unidentified lot) caused water drive in all normal, thyroidectomized and gonadectomized efts within a period of 10 days. Chadwick (1940c, 1941) obtained the migration of efts to water following intramuscular and intraperitoneal implants of hypophyses of a variety of vertebrates such as the water snake (Natrix), the domestic fowl and several genera of urodele and anuran amphibians. That the water drive reaction may be more complex is indicated by Dr. Richard W. Payne (unpublished data) who obtained positive results after the injection of a wide number of pituitary preparations, several of which showed negative prolactin activity by the pigeon crop assay. Tuchmann-Duplessis (1948, 1949) has shown that the administration of 60–120 Riddle-Bates units of prolactin to the land stage of *Triturus cristatus* and *T. alpestris* resulted in the migration of the animals to water and the assumption of pigmentation and morphological characters associated with the aquatic, reproductive phase. The cloaca became enlarged while the gonads and prostate became active. Prolactin administered to castrated males produced only water drive and color changes.

The results reported above indicate that prolactin is probably the active principle concerned with the initiation of water drive and that the thyroid, while not necessary to this reaction, facilitates the process by conditioning normal molting. Nevertheless, the situation remains confused, considering that a number of hormone preparations other than prolactin have produced water drive activity, and it is not clear which of the various phenomena accompanying migration (pigment changes, etc.) are stimulated directly by the water drive factor and which result from endogenous release of other endocrine substances through stimulation of the pituitary. The present investigation is part of an extended study seeking to clarify the complex situation involved in the transformation of the terrestrial eft to the aquatic adult. The use of hypophysectomized test animals has been necessary in order to rule out the endogenous release of prolactin itself by a specific testing agent or non-specific "shock" effect and to eliminate synergic reaction within the pituitary. Grant and Grant (1956) have previously indicated that prolactin causes water migration and skin changes in hypophysectomized efts but none of the other changes toward the adult condition.

"Water drive" is used below to indicate the actual migration of efts to water and "water drive syndrome" to designate migration plus associated morphological changes, etc. The term "drive" is used loosely and is not necessarily meant to imply the operation of precise directive factors.

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Materials and Methods

Efts were collected near Lyme Center, New Hampshire; Quechee, New York; Honesdale, Pennsylvania, and Williamstown, Massachusetts. Animals from different localities were segregated and kept in plastic boxes, 15–20 animals per box, on a thick bed of moss (usually Polytrichum). Room temperature ranged from 21°–24° C. Illumination was that of the room except for a few hours each day when the containers were subjected to direct illumination from an overhead lamp. This procedure aided the growth of the moss, the presence of which seems to be extremely helpful in keeping animals healthy. Attempts made to keep efts on a floor of wet, cellulose sponge proved quite unsuccessful. Animals were fed Enchytraeus worms and blowfly larvae. Some test animals were kept without feeding for extended periods in a refrigerator at approximately 5° C. Hypophysectomized efts are particularly susceptible to infection, but 1% solutions of potassium bichromate or malachite green diluted 1:15,000 have proved efficient prophylactic agents.

The total length of experimental animals ranged from 40 to 74 mm. with weight varying from 0.5 to 1.4 gm. The probable age of such animals was from 1 to 2.5 years and all were well removed from the naturally occurring aquatic phase of their cycle. Snout-vent measurements, which have proved an accurate standard in herpetological work, were also recorded.

Animals were anesthetized in a 0.5% solution of chloretone and hypophysectomized with the aid of a suction pipette attached to an aspiration unit. At the termination of the experiments hypophysectomy was verified histologically on most specimens. Two weeks after hypophysectomy subcutaneous injections were made with a 27-gauge, Huber point needle. In general, injections were made every other day at various concentrations of solution, but a constant volume of 0.1 cc. at each injection was maintained. Water drive responses were studied in containers possessing equal areas of land and water. The time taken for the assumption of the aquatic habitat, the number of molts and the duration of the aquatic phase of life were recorded for all individuals as far as possible.

The various preparations used in the injections were made up in a standard amphibian Ringer's, with controls receiving the same volume of saline as experimental animals. The following pituitary preparations were tested: FSH (swine, Armour Lot No. K45208R), LH (sheep, Armour Lot 227–80), TSH (Armour Lot 317–51), Antuitrin S (Parke Davis Lot P459D), ACTH (hog, Armour Lot K 52204), posterior pituitary preparation (hog, Lot 503), GH (beef growth hormone, Wilhelmi Lot B168), prolactin (Sheep, Schering Lot 4g Hyex 4, Armour sheep Lot 759–CCC and the highly purified sheep preparation of Li), MSH

TABLE 1

Results of water drive studies following the treatment of land phase Diemyctylus viridescens with various pituitary preparations

Treatment	Hypophysectomy	Wt., gm.	Length, mm. Total/Standard	Total dose, mg.	Results	Molting
*FSH Armour**	–	0.55	55/26	8	9 days to water	+
	–	0.80	55/29	8	10 days to water	+
	+	1.20	67/36	8	partial response	abnormal
	+	1.40	69/36	8	8 days to water	abnormal
	+	0.85	67/34	0.8	negative response	–
	+	0.72	63/32	0.8	negative response	–
	+	0.47	56/29	0.8	negative response	–
LH Armour	–	0.81	56/33	8	negative response	–
	–	0.47	48/23	8	negative response	–
	+	1.10	73/36	8	negative response	–
	+	1.12	68/37	8	negative response	–
GH Wilhelmi	+	1.10	73/38	0.8	negative response	+
	+	0.53	60/29	0.8	negative response	–
	+	0.53	59/32	0.8	negative response	–
	+	0.72	63/33	0.8	negative response	–
	+	0.74	63/32	0.8	negative response	–
	+	0.53	51/31	0.8	negative response	–
ACTH Armour	+	0.62	57/26	0.8	negative response	–
	+	0.71	64/32	0.8	negative response	+
	+	0.65	59/32	0.8	negative response	–
	+	0.67	62/34	0.8	negative response	–
	+	0.68	64/33	0.8	negative response	–
A corticotropin (Li)	+	0.84	65/35	0.8	negative response	–
Posterior pituitary Armour	+	1.22	72/37	0.8	negative response	–
	+	0.68	58/32	0.8	negative response	–
	+	0.50	52/31	0.8	negative response	–
	+	0.62	54/31	0.8	negative response	–
TSH Armour	+	0.61	58/32	0.8	negative response	–
	+	0.74	61/34	0.8	negative response	+
	+	0.84	65/35	0.8	negative response	+
	+	0.57	54/33	0.8	negative response	+
	+	0.62	60/33	0.8	negative response	+
Antuitrin S Parke & Davis	–	0.65	52/30	6	dead 7th day	–
	–	0.72	53/35	4	dead 5th day	–
MSH Armour	+	0.88	66/35	2	negative response	–
	+	0.47	51/28	2	negative response	–
	+	0.55	55/30	2	negative response	–
Intermedin (Li)	+	0.60	58/39	0.8	negative response	–

* All animals receiving 8 mg. of FSH showed a tendency toward the olive pigmentation of the adult.

(melanophore stimulating hormone, Armour Lot R 527225). According to Steelman *et al.* (1953), the assay for the FSH preparation shows it to be contaminated with 0.5 I.U. of prolactin per mg., a fact which is of importance in interpreting the results given below.

Results

(*a*) *Various mammalian pituitary preparations*

The detailed results of this series of injections are given in Table I. LH, TSH, ACTH, MSH, posterior pituitary, GH and Antuitrin S failed to induce water drive in any of the animals tested. One animal given 0.8 mg. of ACTH-free Intermedin (Li) also gave a negative response. Most efts treated with TSH underwent a normal molt following injections, while one eft of the GH series and one of the ACTH series showed this reaction. All other preparations failed to produce normal molts in hypophysectomized individuals with the result that the

FIGURE 1. A normal eft (A) is shown beside a hypophysectomized animal (B), which having failed to molt is covered with a thick layer of cornified epithelium.

TABLE II

Results of water drive studies following the treatment of land phase Diemyctylus viridescens with prolactin

Treatment	Wt., gm.	Length, mm. Total/Standard	Total dose, mg.	Effective dose I.U.*	Results	Molting
Non-hypophysectomized *Schering prolactin* 30 I.U./mg.	0.54	50/28	8	240	7 days to water	+
	0.64	51/29	8	240	7 days to water keeling of tail and olive pigmentation	+
Hypophysectomized *Armour prolactin* 25–30 I.U./mg.	1.12	74/39	8	216	8 days to water	abnormal
	0.41	56/29	8	162	6 days to water	–
	0.61	63/33	0.8	23	10 days to water	abnormal
	0.43	55/28	0.8	23	8 days to water	–
	0.76	57/31	0.8	23	7 days to water	abnormal
	0.80	60/29	0.08	—	partial response	abnormal
Hypophysectomized *Prolactin (C.H.Li)* 35 I.U./mg.	0.66	57/32	0.4	14	10 days to water	–
	1.14	69/36	0.4	10.5	5 days to water	–
	0.63	59/32	0.4	10.5	5 days to water	abnormal
	0.75	65/34	0.4	7	4 days to water	abnormal
	0.59	58/32	0.4	10.5	5 days to water	–
	0.48	55/29	0.4	—	Dead on 3rd day	
	0.73	60/32	0.4	—	Dead on 4th day	
	0.80	62/34	0.4	14	7 days to water	–
	0.71	57/31	0.4	14	8 days to water	abnormal
	0.35	40/26	0.4	14	7 days to water	abnormal
	0.60	57/30	0.4	14	7 days to water	–
	0.61	54/29	0.4	14	8 days to water	abnormal
	0.97	69/36	0.4	10.5	6 days to water	abnormal
Hypophysectomized *Prolactin (C.H.Li)* 35 I.U./mg.	0.87	58/34	0.04	1.4	8 days to water	–
	0.92	64/35	0.04	1.4	10 days to water	–
	0.63	57/29	0.04	1.4	8 days to water	abnormal
	0.82	65/34	0.04	1.4	8 days to water	–
	0.44	50/27	0.04	1.4	8 days to water	abnormal
	0.78	70/35	0.04	1.05	6 days to water	–

* Effective dose is estimated as the amount of prolactin efts had received at the time of their assumption of an aquatic habitat.

efts rapidly became covered with a thick, black layer of cornified epithelium until even the eyes were obscured (Fig. 1).

Both normal and hypophysectomized efts receiving a total of 8 mg. of FSH showed water drive activity from 8 to 10 days following the initial injections. One animal failed to give a complete reaction and was in and out of water for several weeks before returning permanently to land. In these animals molting was abnormal with the skin sloughing off in irregular patches after the efts had entered water. There was a trend in the pigmentation of all individuals toward the adult olive, though this was more marked in the non-operated efts. It is interesting that tests for water drive in animals receiving 0.8 mg. of FSH were completely negative, and that molting failed to occur.

(*b*) *Tests with prolactin*

Two unhypophysectomized animals receiving 8 mg. (240 I.U.) of Schering prolactin migrated to water on the seventh day following the initial injections and within a few weeks had acquired many features associated with the water drive syndrome (*i.e.,* smooth, moist skin, olive pigmentation and keeling of the tail). Other prolactin preparations were injected into hypophysectomized efts in doses varying from 8.0 to 0.04 mg. as shown in Table II. In all cases where death did not occur before the injections were completed, the tests were positive. The animals assumed the aquatic habitat from 4 to 10 days following the first injection. It should be noted that several animals migrated before all injections had been completed and it is therefore desirable to give results in terms of the effective dose (*i.e.,* the dose animals had received at the time of the water-drive response) rather than total dose. The range in effective dose was from 216 to 1.05 I.U.

The water drive reaction is very positive as animals giving the reaction remain completely submerged, take food under water and will immediately return to water if placed on land. One eft receiving 0.08 mg. (2.3 I.U.) failed to give the complete reaction but migrated alternately between land and water over the time observed. Records on the duration of water drive are far from complete as most test animals died before leaving water. However, in a number of cases animals actually did return to land after periods varying from two to five weeks.

It is of particular significance to note that whereas all hypophysectomized animals showed positive water drive in response to treatment with prolactin, they failed to assume the olive pigmentation and tail keel associated with the water drive syndrome. When molting occurred it was abnormal, but beneath the patches of thickened corneum the smooth, moist skin retained the orange pigmentation of the eft and showed no tendency toward the adult olive. No keeling of the tail was apparent in any individual even after extended periods in water.

Conclusions

The primary concern of the present investigation was to determine as precisely as possible the endocrine factor responsible for water drive in the red eft. From the results reported above we are in agreement with Chadwick (1940c) that prolactin is the active principle. All animals treated with this substance migrated to water and assumed a smooth skin texture similar to that of the adult. As tests were conducted on hypophysectomized efts the possibility of hypophyseal synergy or endogenous release must be ruled out. All other pituitary preparations administered gave a negative reaction with the exception of the 8-mg. dose of FSH. This is understandable, as the assay for the gonadotropin shows it to be contaminated with lactogenic hormone. Both the Armour prolactin and the homogeneous preparation of Li produced positive results in animals receiving as little as 2.3 to 1.4 I.U. per effective dose. The 4 I.U. of prolactin contained in the FSH were therefore quite sufficient to initiate water drive. No minimum dosage level for the water drive reaction has yet been established but the failure of 0.8 mg. FSH to elicit positive results may indicate it to be about 0.4 I.U. Though there was some variability in the time animals responded to treatment with prolactin, there is at present no indication of a dose-response relationship and it is suggested that the reaction may follow the all-or-none principle.

Reinke and Chadwick (1940) have shown that the thyroid and gonads are not directly involved in the water drive response and initial histological survey of our prolactin tests shows no thyrotropic or gonadotropic activity. The lactogenic hormone is not effective in promoting molt in hypophysectomized animals as it was in normal efts studied by Chadwick and Jackson (1948). However, as molting did occur in efts receiving injections of TSH it suggests that the increased molting reported by Chadwick and Jackson (1948) in intact animals was due to the endogenous release of TSH resulting from treatment.

Though our investigations are in general agreement with those of Chadwick, we cannot support his assumption that prolactin effects the entire water drive syndrome. Work on hypophysectomized animals indicates that the problem is considerably more complex and can tentatively be divided into four major steps.

1. Migration to water and change of skin texture: induced by prolactin.
2. Normal molting which facilitates but does not directly affect water drive: release of thyroid hormone mediated through the pituitary (TSH).
3. Appearance of olive pigmentation: unknown principle involved, possibly MSH.
4. Morphological characteristics associated with water drive such as keeling of the tail and development of the lateral line system: unknown principle or principles involved.

It is tempting to suggest that prolactin may initiate the entire water drive syndrome by triggering the endogenous release of other endocrines which induce many changes associated with the aquatic phase. The identification of these substances, the parts of the cycle they effect and possible interrelationships involved will be taken up in future papers. In conclusion it appears safe to say that the lactogenic hormone produces water drive and skin change, and that the red eft test for the presence of prolactin (1 I.U. or above) is a positive and reliable one.

Summary

1. Other investigators have shown that the land (eft) stage of *Diemyctylus viridescens* can be induced to enter water and assume adult pigmentation and morphological characteristics following treatment with various pituitary preparations. Hypophysectomized efts were used in the present experiment in order to assure positive identification of the active, water drive principle.

2. Operated animals treated with LH, growth hormone, ACTH, posterior pituitary preparation, TSH, Antuitrin S and melanophore-stimulating hormone gave a negative response. Eight-milligram injections of FSH produced water drive in

several animals, but this was most probably due to the contamination of the substance with prolactin.

3. Most hypophysectomized efts, with the exception of those receiving TSH, either failed to molt or underwent an abnormal molt after the animals had been induced to enter water.

4. Operated animals receiving injections of prolactin (240 to 1.05 I.U.) migrated to water from 4 to 10 days following treatment. However, they failed to acquire adult pigmentation and associated characteristics.

5. The lactogenic hormone has been identified as the principle which initiates the migration of efts to water and the water drive test for prolactin is considered to be reliable.

LITERATURE CITED

Adams, A. E., 1932. Observations on the effect of anterior pituitary extract (phyone) on the 'red phase' of *Triturus viridescens*. *Anat. Rec.*, **52**: 46. (Abst.)

Adams, A. E., and M. C. Grierson, 1932. Cornification and molting in *Triturus*. *Proc. Soc. Exp. Biol. Med.*, **30**: 341–344.

Adams, A. E., A. Kuder and L. Richards, 1932. The endocrine glands and molting in *Triturus viridescens*. *J. Exp. Zool.*, **63**: 1–55.

Chadwick, C. S., 1940a. Induction of water drive in *Triturus viridescens* with anterior pituitary extract. *Proc. Soc. Exp. Biol. Med.*, **43**: 509–511.

Chadwick, C. S., 1940b. The water drive in *Triturus viridescens* as an effect of the growth promoting hormone of the anterior hypophysis. *J. Tenn. Acad. Sci.*, **15**: 412.

Chadwick, C. S., 1940c. Identity of prolactin with water drive factor in *Triturus viridescens*. *Proc. Soc. Exp. Biol. Med.*, **45**: 335–337.

Chadwick, C. S., 1941. Further observations on the water drive in *Triturus viridescens*. II. Induction of the water drive with the lactogenic hormone. *J. Exp. Zool.*, **86**: 175–187.

Chadwick, C. S., 1948. Evidence of a thyroid-skin gland relationship in the induction of molting in the red eft of *Triturus viridescens*. *Anat. Rec.*, **101**: 678. (Abst.)

Chadwick, C. S., and H. R. Jackson, 1948. Acceleration of skin growth and molting in the red eft of *Triturus viridescens* by means of prolactin injections. *Anat. Rec.*, **101**: 718. (Abst.)

Dawson, A. B., 1936. Changes in the lateral line organs during the life of the newt *Triturus viridescens*. A consideration of the endocrine factors involved in the maintenance of differentiation. *J. Exp. Zool.*, **74**: 221–237.

Grant, W. C., and J. A. Grant, 1956. The induction of water drive in the land stage of *Triturus viridescens* following hypophysectomy. *Anat. Rec.*, **125**: 604. (Abst.)

Noble, G. K., 1926. The Long Island newt; a contribution to the life history of *Triturus viridescens*. *Amer. Mus. Nov., No. 228.*

Noble, G. K., 1929. Further observations on the life-history of the newt *Triturus viridescens*. *Amer. Mus. Nov., No. 348.*

Reinke, E. E., and C. S. Chadwick, 1939. Inducing land stage of *Triturus viridescens* to assume water habitat by pituitary implants. *Proc. Soc. Exp. Biol. Med.*, **40**: 691–693.

Reinke, E. E., and C. S. Chadwick, 1940. The origin of water drive in *Triturus viridescens*. I. Induction of the water drive in thyroidectomized and gonadectomized land phases by pituitary implantations. *J. Exp. Zool.*, **83**: 224–233.

Steelman, S. L., W. A. Lamont, W. A. Dittman and E. J. Hawrylewicz, 1953. Fractionation of the swine follicle stimulating hormone. *Proc. Soc. Exp. Biol. Med.*, **82**: 645–647.

Tuchmann-Duplessis, H., 1948. Développement des caractères sexuels du Triton traité par des hormones hypophysaires gonadtropes et lactogènes. *C. R. Soc. Biol.*, **142**: 629–630.

Tuchmann-Duplessis, H., 1949. Action de l'hormone gonadtrope et lactogène sur le comportment et les caractères sexuels secondaires du triton normal et castré. *Arch. Anat. Micr. Morph. Exp.*, **38**: 302–317.